codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 7 (a) **CX/PR 09/41/5**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON PESTICIDE RESIDUES
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PROPOSED DRAFT REVISION OF THE GUIDELINES ON THE ESTIMATION OF UNCERTAINTY OF RESULTS FOR THE DETERMINATION OF PESTICIDE RESIDUES (CAC/RCP 59-2006) AT STEP 3

Governments and interested international organizations are invited to submit comments on the above subject matter at Step 3 (See Appendix) and should do so in writing in conformity with the Uniform Procedure for the Elaboration of Codex Standards and Related Texts (see *Procedural Manual of the Codex Alimentarius Commission, Seventeenth Edition*) **to**: Mr Josef Brodesser, Food and Environmental Protection Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, P.O. Box 100, Wagramer Strasse 5, Tel: +43-1-2600-26058, FAX +43-1-26007, or email j.brodesser@iaea.org with copies **to**: 1. Secretary, Codex Alimentarius Commission, Joint WHO/FAO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy, by email codex@fao.org or fax: +39-06-5705-4593 and 2. Duang Lifang, Engineer, Institute for the Control of Agrochemicals, Ministry of Agriculture, P.R China, Fax: +0086 10 64194064, email: ccpr@agri.gov.cn **by 15 February 2009.**

Background

The last 40th Session of the Codex Committee on Pesticide Residues had considered the discussion paper which had been prepared at the request of the 39th session of the Committee as a basis for a guidance document on the estimation of measurement uncertainty. The Representative of IAEA recalled that estimation of measurement uncertainty for multi-residue methods was problematic for many laboratories, and noted that when applying the "bottom-up" mathematical model calculation, the application of existing Guidelines such as ISO Guide 2 and Eurachem GUM was very complicated and time consuming.

The Committee noted that the working group had supported the development of guidance on the estimation of measurement uncertainty on the basis of the empirical approach ("top down") and had discussed the relationship between the work on pesticide residue analysis and the work of the Committee on Methods of Analysis and Sampling. The Representative of IAEA recalled that CCMAS addressed measurement uncertainty from a general perspective and did not specifically consider matters related to pesticide residue analysis, but was kept informed of the work of the CCPR in order to ensure consistency throughout Codex. The Committee was also informed that the last session of the CCMAS had proposed new work on the revision of the Guidelines on

Measurement Uncertainty (CAC/GL 54-2004) in order to provide additional guidance in this area.

Several delegations supported the development of guidance on measurement uncertainty in pesticide residue analysis in view of the difficulties faced by laboratories, especially in developing countries, and indicated that they also applied empirical calculations of uncertainty at the national level. Some delegations pointed out that the differences in approach between national authorities on the use of measurement uncertainty for enforcement purposes could create trade problems.

The Committee agreed to propose new work on the revision of the Guidelines on the Estimation of Measurement Uncertainty (CAC/GL 59-2006) and the 31st Session of the Commission had approved this new work (Job Code N13-2008).

The Committee further agreed that an electronic working group coordinated by IAEA, open to all members and observers and working in English, would prepare a Proposed Draft Revision of the Guidelines in order to provide practically oriented recommendations including examples on the estimation of measurement uncertainty and application of the concept for pesticide residue laboratories, as described in the project document. The Committee agreed that examples should be included in the guidance document in order to facilitate the better understanding of the estimation of measurement uncertainty by residue testing laboratories.

Member governments and interested international organizations are invited to provide their comments to addresses indicated above by 15 February 2009 (see Appendix below).

Appendix

GUIDELINES ON ESTIMATION OF UNCERTAINTY OF RESULTS

CAC/GL 59-2006

1. INTRODUCTION

It is a requirement under ISO/IEC 17025 that laboratories determine and make available the uncertainty associated with analytical results. To this end, food testing laboratories operating under Revised Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40-1993, Rev. 1- 2003) should have available sufficient data derived from method validation/verification, inter-laboratory studies and in-house quality control activities, which can be applied to estimate the uncertainties particularly for the routine methods undertaken in the laboratory. These guidelines were prepared taking into account the general recommendations of the CCMAS.

1.1 CONCEPT AND COMPONENTS OF UNCERTAINTY

Measurement uncertainty refers to the 'uncertainty' associated with data generated by a measurement process. In analytical chemistry, it generally defines the uncertainty associated with the laboratory process but may also include an uncertainty component associated with sampling.

The uncertainty 'estimate' therefore describes the range around a reported or experimental result within which the true value can be expected to lie within a defined level of probability. This is a different concept to measurement error which can be defined as the difference between an individual result and the true value. The reporting of uncertainty is intended to provide a higher level of confidence in the validity of the reported result.

Contributions to data uncertainty are manifold and described in detail in Tables 1and 2. The evaluation of uncertainty ideally requires an understanding and estimation of the contributions to the uncertainty of each of the activities involved in the measurement process.

2. IDENTIFICATION OF UNCERTAINTY SOURCES

In general, the uncertainty of measurements is comprised of many components, arising from activities involved with the sample. The uncertainty of an analytical result is influenced by three major phases of the determination:

- External operations: sampling (S_S), packing, shipping and storage of samples[1];
- Preparation of test portion: sub-sampling, sample preparation and sample processing (S_{Sp});
- Analysis (S_A): extraction, cleanup, evaporation, derivatisation, instrumental determination[2]

The combined standard (S_{Res}) and relative (CV_{Res}) uncertainty may be calculated according to the error propagation law:

$$S_{\mathrm{Re}s} = \sqrt{S_S^2 + (S_{Sp}^2 + S_A^2)} \;;\; S_{\mathrm{Re}s} = \sqrt{S_S^2 + S_L^2} \qquad (1)$$

If the whole sample is analysed, the mean residue remains the same and the equation can be written

[1] Packing, shipping, storage, and laboratory preparation of samples may have significant influence on the residues detected, but their contribution to the uncertainty can often not be quantified based on the current information. Examples of such errors are e.g, selection of sampling position, time of sampling, Incorrect labelling decomposition of analytes or contamination of the sample

[2] If the result has been corrected for the recovery, the uncertainty associated with this correction shall be incorporated.

as:

$$CV_{Res} = \sqrt{CV_S^2 + CV_L^2} \text{ and } CV_L = \sqrt{CV_{Sp}^2 + CV_A^2} \quad (2)$$

Where CV_L is the relative uncertainty of the laboratory phase of the determination which may derive from the sub-sampling, sample preparation, sample processing and analytical steps.

It should be noted that a laboratory is normally only required to estimate the uncertainty associated with those processes for which it has control, that is, only those processes that take place in the laboratory if sampling is not the responsibility of the laboratory staff.

2.1 ERRORS IN ANALYTICAL MEASUREMENTS

In most measurements we can distinguish between three types of errors: gross, random and systematic errors.

Gross errors refer to unintentional/unpredictable errors while generating the analytical result. Errors of this type invalidate the measurement. Laboratory quality assurance procedures should minimize gross errors. It is not possible or desirable to statistically evaluate and include the gross errors in the estimation of uncertainty. They need no further discussion in this document.

Random errors are present in all measurements, and cause replicate results to fall on either side of the mean value. The random error of a measurement cannot be compensated for, but increasing the number of observations and training of the analyst may reduce the effects.

Systematic errors occur in most experiments, but their effects are quite different. The sum of all the systematic errors in an experiment is referred to as the bias. Since they do not sum to zero over a large number of measurements, individual systematic errors cannot be detected directly by replicate analyses. The problem with systematic errors is that they may go undetected unless appropriate precautions are taken. In practice, systematic errors in an analysis can only be identified if the analytical technique is applied to a reference material, the sample is analysed by another analyst or preferably in another laboratory, or by re-analysing the sample by another analytical method. However, only if the reference material matches identically in terms of analyte, matrix, and concentration does it meet the ideal conditions for determining the bias of the method. The bias of a method may also be investigated by recovery studies. However, recovery studies assess only the effects of analysis (S_A) and do not necessarily apply to naturally incurred samples, or components of the bias that may be introduced prior to the analytical step. In pesticide analysis, results are not normally corrected for the recovery, but should be corrected if the average recovery is significantly different from 100%. If the result has been corrected for recovery, the uncertainty associated with recovery should be incorporated in the uncertainty estimation of the measurement.
Some examples of sources of errors are illustrated in Tables 1 and 2 It should be noted that not all sources mentioned have to be evaluated in the uncertainty estimation. Some sources are already incorporated in the overall uncertainty, while others are negligible and may be disregarded. However, it is important to recognise and assess all sources before elimination. Further information may be obtained from published documents[3,4].

[3] EURACHEM Guide to Quantifying Uncertainty in Analytical Measurements, 2nd ed. 1999, http://www.measurementuncertainty.org
[4] Ambrus A. Reliability of residue data, Accred. Qual. Assur. 9, pp. 288-304. 2004.

Table 1: Sources of error in preparation of the test portion

	Sources of systematic error	Sources of random error
Sample preparation	The portion of sample to be analysed (analytical sample) may be incorrectly selected	The analytical sample is in contact and contaminated by other portions of the sample
		Rinsing, brushing is performed to various extent, stalks and stones may be differentially removed
Sample processing (S_{Sp})	Decomposition of analyte during sample processing, cross contamination of the samples	Non homogeneity of the analyte in single units of the analytical sample
		Non homogeneity of the analyte in the ground/chopped analytical sample
		Variation of temperature during the homogenisation process
		Texture (maturity) of plant materials affecting the efficiency of homogenisation process

Table 2: Sources of error in analysis (S_A):

	Sources of systematic error	Sources of random error
Extraction/Cleanup	Incomplete recovery of analyte	Variation in the composition (e.g. water, fat, and sugar content) of sample materials taken from a commodity
	Interference of co-extracted materials (load of the adsorbent)	Temperature and composition of sample/solvent matrix
Quantitative determination	Interference of co-extracted compounds	Variation of nominal volume of devices within the permitted tolerance intervals
	incorrect purity of analytical standard	Precision and linearity of balances
	Biased weight/volume measurements	Incomplete and variable derivatisation reactions
	Operator bias in reading analogue instruments, equipment	Changing of laboratory-environmental conditions during analysis
	Determination of substance which do not originate from the sample (e.g. contamination from the packing material)	Varying injection, chromatographic and detection conditions (matrix effect, system inertness, detector response, signal to noise variation etc.)
	Determination of substance differing from the residue definition	Operator effects (lack of attention)
	Biased calibration	Calibration

3. PROCEDURES FOR ESTIMATING MEASUREMENT UNCERTAINTY

Whilst there are a number of options available to laboratories for the estimation of measurement uncertainty, there are two procedures described as the 'bottom up' approach and the 'top down' approach[1] that are the most commonly used.

The bottom-up method:

The bottom up or component-by-component approach incorporates an activity-based process whereby the analyst breaks down all the analytical operations into primary activities. These are then combined or grouped into common activities and an estimate made of the contribution of these activities to the combined uncertainty value of the measurement process. The bottom up approach can be very laborious and requires a detailed knowledge of the whole analytical process. The benefit to the analyst is that this approach provides a clear understanding of the analytical activities which contribute significantly to the measurement uncertainty and which therefore may be assigned as critical control points to reduce or manage measurement uncertainty in future applications of the method.

The top-down method:

The top down approach is based on method validation and long-term precision data derived from laboratory control samples, proficiency testing results, published literature data and/or inter-laboratory collaborative trials. Uncertainty estimates based on inter-laboratory studies may also take into account the between-laboratory variability of the data and provides a reliable estimate of the method performance and the uncertainty associated with its application. It is important to acknowledge however that collaborative studies are designed to evaluate the performance of a specific method and participating laboratories. They normally do not evaluate imprecision due to sample preparation or processing as the samples generally tend to be highly homogenized.

Pesticide residue analytical laboratories normally look for over 200 residues in numerous commodities that lead to practically infinite number of combinations. Therefore it is suggested that, for estimating the uncertainty associated with multi residue procedures, laboratories use a properly selected range of analytes and sample matrices which represents the residues and commodities to be analysed in terms of physical chemical properties and composition according to the relevant parts of the *Revised Guidelines on Good Laboratory Practice* rather than establishing the uncertainty for each method/analyte/matrix combination. The selection of a representative range of analytes and matrices to provide an uncertainty estimate should be supported by validation data and studies on the selected matrix / analyte combination.

In summary, laboratories should use either their own long-term precision data or the activity-based procedure (component by component calculation) to establish and refine the uncertainty data.

In certain situations it may also be appropriate to estimate the uncertainty contribution due to sample variability. This will require an understanding of the analyte variability within the sample lot and is not readily available to the laboratory or the analyst The values obtained from the statistical analysis of over 8500 residue data (Table 4) provide currently the best estimate[5]. These estimates can be incorporated into the combined uncertainty value.
Likewise it may be necessary to take into consideration the stability of analytes during sample storage and processing if these are likely to result in analyte variability between analysts and laboratories.

3.1 UNCERTAINTY ESTIMATES OF RESULTS INVOLVING ANALYSIS OF MULTI-COMPONENTS

The estimation of uncertainty of results for multi-component residues arising from the application of technical mixtures including structural and optical isomers, metabolites and other breakdown

[5] Ambrus A and Soboleva E. Contribution of sampling to the variability of residue data, JAOAC. 87, 1368-1379, 2004.

products may require a different approach particularly where the MRL has been established for the sum of all or some of the component residues. The assessment of the random and systematic errors of the results based on the measurements of multiple peaks is explained in detail in a recent publication[6].

4. GUIDANCE VALUES FOR ACCEPTABLE UNCERTAINTIES

The establishment of the standard deviation of a series of tests ran by a single laboratory, as a measure of standard uncertainty, requires the results a large data-set that is not always available. However, for smaller amounts of data the true standard deviation can be estimated as follows:

Depending on the number of observations (n), the relation of the true (σ) standard deviations, calculated (S) standard deviations, and the expected range of the mean value ($\bar{x}$) at 95% probability are illustrated in Table 3. The multiplying factor, ***f***, provides the link between the estimated and true values as the function of the number of measurements.

Table 3 The values of *f* for calculation of expected ranges of standard deviation and mean values

N	$S_{min}=f_1\sigma$	$S_{max}=f_2\sigma$	$\bar{x} = \pm f_3 S$
	f_1	f_2	f_3
5	0.35	1.67	1.24
7	0.45	1.55	0.92
15	0.63	1.37	0.55
31	0.75	1.25	0.37
61	0.82	1.18	0.26
121	0.87	1.13	0.18

For instance: the repeatability of the laboratory operations, CV_L, was determined from 5 test portions drawn from a homogenised sample containing incurred residues. The average residue found was 0.75 mg/kg with a standard deviation of 0.2 mg/kg. The true residue of the processed sample can be expected between 0.75± 1.24*0.2 = 0.75±0.248 mg/kg, while the true uncertainty of the measurement results is likely to be between 0.0696 (0.2*0.35) and 0.334 (0.2*1.67) mg/kg in 95% of the cases.

The guidance values for standard uncertainty, given in Table 4, are based on a large number of data and can be used to assess the reality of the estimated uncertainty in a laboratory in order to avoid an unreasonable high or low value.

Table 4. Typical expected uncertainties of major steps in the sampling and analysis of pesticide residues

Procedure	**Relative uncertainty**	**Comments**
Sampling of commodities of plant origin. Reflects the variation of	Medium and small commodities. (Sample size ≥10)[a]: 26-30%[b]	For testing compliance with MRLs, the sampling uncertainty is defined as 0, as the MRLs

[6] Soboleva E., Ambrus A., Jarju O., Estimation of uncertainty of analytical results based on multiple peaks, J. Chromatogr. A. 1029. 2004, 161-166

Procedure	Relative uncertainty	Comments
mean residues being in composite samples taken randomly from a lot. It does not incorporate the errors of follow-up procedures.	Large commodities. (Sample size ≥5)[a]: 36-40%[b]	refer to the average residues in bulk samples.
Sampling of animal products	The relation between the number of samples (n) to be taken for detection of a specified percentage of violation (β_p) with a given probability (β_t), is described by[a]: $1-\beta_t = (1-\beta_p)^n$	The primary samples should be selected randomly from the whole lot.
Sample processing Includes the physical operation performed for homogenizing the analytical sample and subsampling , but excludes decomposition and evaporation of analytes.	Largely varying depending on sample matrix and equipment. No typical value can be given. The analysts should try to keep it[c] below 8-10%.	It may be influenced by the equipment used for chopping / homogenising the sample and the sample matrix, but it is independent from the analyte.
Analysis It includes all procedures performed from the point of spiking of test portions.	Within laboratory reproducibility: 16-53% for concentrations of 1µg/kg to 1 mg/kg[c]. Average between-laboratories reproducibility within 0.001-10 mg/kg: 25%[d]	The typical CV_A can be conveniently determined from the recovery studies performed with various pesticide-commodity combinations on different days and during the use of the method.

Notes:

(a) Recommended Method of Sampling for the Determination of Pesticide Residues for Compliance with MRLs, (CAC/GL 38-1999).

(b) Ambrus A. Soboleva E. Contribution of sampling to the variability of residue data, JAOAC, 87, 1368-1379, 2004;

(c) Guidelines on Good Laboratory Practice in Residue Analysis (CAC/GL 40-1993, Rev. 1-2003)

(d) Alder L., Korth W., Patey A., van der Schee and Schoeneweis S., Estimation of Measurement Uncertainty in Pesticide Residue Analysis, J. AOAC International, 84, 1569-1578, 2001

In addition to the estimated uncertainties made by the individual laboratories, regulatory authorities and other risk managers may decide on a default expanded uncertainty of measurements which can be used in judging compliance with MRLs (See section 5) based on between-laboratories reproducibility values. For instance, a 50% expanded uncertainty for CV_L is considered to be a reasonable default value.

5. USE OF UNCERTAINTY INFORMATION

If required, the result should be reported together with the expanded uncertainty, U, as follows

Result = $x \pm U$ (units)

The expanded uncertainty, U, may be calculated from the standard combined uncertainty (S_{Res}) with a coverage factor of 2 as recommended by EURACHEM or with the Student ***t*** value for the level of confidence required (normally 95%) where the effective degree of freedom is less than 20. The respective calculations for the expanded uncertainty are as follows

$$U = 2S_{Res} \quad \text{or} \quad U = t_{v,0.95}S_{Res} \qquad (3)$$

The numerical value of the reported results should follow the general rule that the last digits can be uncertain. Rounding the results should be done only when the final result is quoted since rounding at the initial stages of calculation may introduce unnecessary bias in the calculated values.

For the purpose of explication, it is assumed that the best estimate of the residue content is reported for a sample. How the results are interpreted depends upon the purpose of the testing. Typical reasons include testing compliance with the national MRL, certifying compliance with the Codex MRL of a commodity for export.

5.1 Testing compliance with an MRL

Figure 1 shows how the testing results can be displayed in terms of the measured value of the residue, the corresponding uncertainty interval, and the MRL.

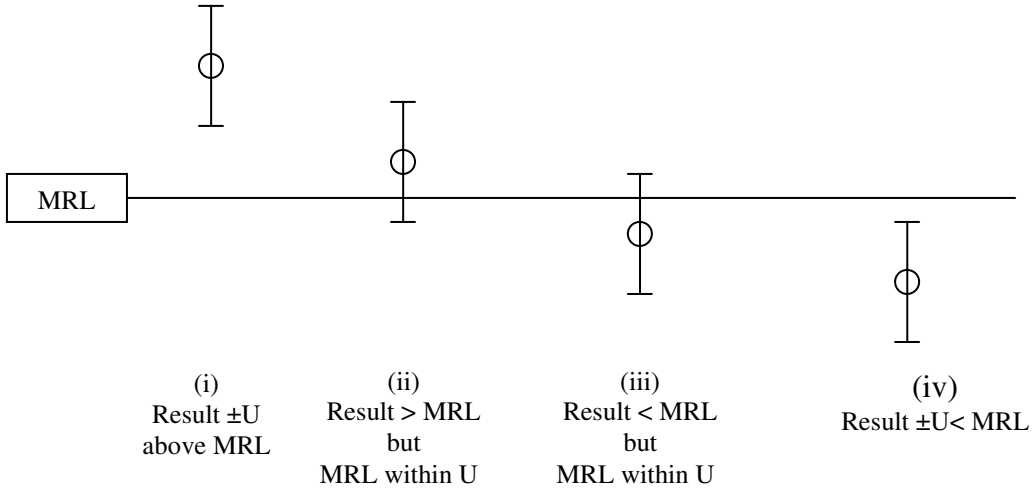

Figure 1. Illustration of the relationship of measured value expected uncertainty and MRL

Situation (i)

The analytical result bounded by the measurement uncertainty endpoints is greater than the MRL. The result indicates that the residue in the sampled lot is above the MRL.

Situation (ii)

The analytical result is greater than the MRL with the lower endpoint of the measurement uncertainty less than the MRL

Situation (iii)

The analytical result is less than the MRL with the upper endpoint of the measurement uncertainty being greater than the MRL.

Situation (iv)

The analytical result bounded by the expanded measurement uncertainty endpoints is less than the MRL.

5.2 Decision Environment

The situations illustrated in Figure 1 are relevant for products of plant origin. The compliance of residues with MRLs for animal products should be decided following sampling plans based on distribution free statistics and examples given in the document on Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs (CAC/GL 33-1999).

Since the residues in every sample that concurs with the minimum sample size and sample mass specified in the Codex Sampling Procedure should comply with the MRL, the expanded uncertainty should be calculated using SL from equation 1 as U = kSL.where SL= CVL* residue.

The decision-making in Situation (i) is clear. In order to avoid lengthy explanation of the uncertainty involving the performance of the analysis for testing compliance with the MRL at the national

level in locally produced or imported commodities, the laboratory may report the results as the sample contains "not less than 'x – U' residues." This satisfies the requirement that the MRL was exceeded beyond any reasonable doubt accounting for measurement uncertainty.

In situation (iv) the sample is clearly compliant with the MRL.

In situations (ii) and (iii) it cannot be concluded that the MRL is exceeded or compliant without reasonable doubt. Action by decision makers may need further consideration as discussed below.

The implications of situations (ii) and (iii) will depend on national practices and may have considerable impact on the acceptance of trade consignments. Caution should be exercised in distributing products in domestic markets or international trade with test results illustrated in situations (ii) and (iii). For example when certifying products for export it may not be advisable to export consignments with residue results as described in situations (ii) and (iii). For countries importing commodities with residue levels as described in situation (ii) it may be difficult to verify compliance with the MRL with an acceptable level of confidence. Situation (iii) generally may not lead to actions by the importing party.

Glossary of terms used in the text[a]

Blank (sample, reagent)	(i) Material (a sample, or a portion or extract of a sample) known not to contain detectable levels of the analyte(s) sought. Also known as a matrix blank. (ii) A complete analysis conducted using the solvents and reagents only, in the absence of any sample (water may be substituted for the sample, to make the analysis realistic). Also known as a reagent blank or procedural blank.
Combined standard uncertainty	For a measurement result, y, the total uncertainty, $u_c(y)$ is an estimated standard deviation equal to the positive square root of the total variance obtained by combining all uncertainty components using the law of propagation of uncertainty (error propagation law)
Contamination	Unintended introduction of the analyte into a sample, extract, internal standard solution etc., by any route and at any stage during sampling or analysis.
Residue definition	The definition of a residue is that combination of the pesticide and its metabolites, derivatives and related compounds to which the MRL applies or which is used for dietary exposure assessment.
Determination system	Any system used to detect and determine the concentration or mass of the analyte. For example, GC-FPD, LC-MS/MS, LC with post-column derivatisation, ELISA, TLC with densitometry, or bioassay.

Level	In this document, refers to concentration (e.g. mg/kg, µg/ml) or quantity (e.g. ng, pg).
Lot	A quantity of a food material delivered at one time and known, or presumed, by the sampling officer to have uniform characteristics such as origin, producer, variety, packer, type of packing, markings, consignor, etc.
Matrix effect	An influence of one or more undetected components from the sample on the measurement of the analyte concentration or mass. The response of some determination systems (e.g. GC, LC-MS, ELISA) to certain analytes may be affected by the presence of co-extractives from the sample (matrix).
Procedural blank	See blank.
Reagent blank	See blank.
Response	The absolute or relative signal output from the detector when presented with the analyte.
Spike or spiking	Addition of analyte for the purposes of recovery determination or standard addition.
Standard uncertainty	Expressed as the standard deviation of an uncertainty component.
Unit (as part of sample)	A single fruit, vegetable, animal, cereal grain, can, etc. For example, an apple, a T-bone steak, a grain of wheat, a can of tomato soup.
Violative residue	A residue which exceeds the MRL or is unlawful for any other reason.

Note (a). The definitions given are based on the following references[7,8,9,10]. Additional definitions are given in the revised GLs on Good laboratory Practice in Residue Analysis[11].

[7] EURACHEM (2000) EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurements 2nd ed. http://www.measurementuncertainty.org

[8] Codex Secretariat. Recommended method of sampling for the determination of pesticide residues for compliance with MRLs, ftp://ftp.fao.org/codex/standard/en/cxg_033e.pdf

[9] Willetts P, Wood R (1998) Accred Qual Assur 3: 231-236

[10] , International Vocabulary of basic and general terms in Metrology, Geneva 1993

[11] Report of the 35th Session of CCPR Appendix VI